REMARKS/ARGUMENTS

Petition is hereby made under the provisions of 37 CFR 1.136(a) for an extension of three months of the period for response to the Office Action. The enclosed cheque includes the prescribed fee.

Having regard to applicants response to the restriction requirement, the Examiner withdrew claims 11 to 23 from consideration as being directed to a non-elected invention. These claims now have been cancelled, such cancellation being without prejudice to applicants right to file a divisional or continuation application directed thereto.

The Examiner noted that the drawings were objected to under 37 CFR 1.84 on the PTO-948 attached to the Office Action. Remedial action with respect thereto can await allowance of this application.

The Examiner noted that there exist several copending applications related to this case, citing as examples Applications Nos: 08/621,944, 08/483,855 and 08/945,567. The Examiner also may wish to note Application No. 08/431,718 (now USP 6,335,018). The Examiner requested a copy of the pending or allowed claims from such applications to consider possible double patenting issues. A copy of each set of claims is enclosed in compliance with the Examiner request.

The Examiner objected to the specification as utilizing trademarks. The disclosure has been amended to acknowledge the trademark nature of the terms involved. It is submitted that the specification is no longer objectionable in this regard.

The Examiner rejected claims 1 to 3 and 9 under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention.

In this regard, the Examiner considered claims 1 and 3 to be indefinite with respect to recitation of certain Figures of the drawings. While not agreeing with the Examiner, in the interests of expediting prosecution, claims 1 and 3 now refer to

SEQ ID Nos, rather than Figures. With respect to claims 7 and 8, the references to Figures 10 and 19 have been retained, for the reasons discussed below.

With respect to claim 2, Table 1A shows the list of strains of *M. catarrhalis* examined by applicants for the expression of a 200 kDa protein, identified by + indication in the right hand column. Since claim 1 refers specifically to strains 4223, Q8 and LES-1, there are excluded from the strains claimed in claim 2. In addition, strains not expressing a 200 kDa protein (- indication in right hand column) are not included. The language of claim 2, therefore, is very clear as to the strains included within the scope of claim 2.

The Examiner considered claim 9 to be vague and indefinite in reciting "an approximately C-terminal half" of the full length protein. It is submitted that it is clear that the molecule has approximately half the size of the full length protein and is a C-terminal fragment. It is submitted that the language is clear in scope.

The Examiner considered claim 3 to be indefinite in referring to "capable of expressing". The Examiner's kind suggestion for revision has been adopted.

Having regard to the above and the revisions made to the claims, it is submitted that claims 1 to 3 and 9 are no longer be open to rejection under 35 USC 112, second paragraph, and hence the rejection should be withdrawn.

The Examiner rejected claims 7 and 8 under 35 USC 112, first paragraph, as failing to provide an enabling disclosure, because the specification does not provide evidence that the claimed biological materials are (1) known and readily available to the public; (2) reproducible from the written description; or (3) deposited.

Claim 7 refers to vectors pKS348 and pKS294, both of which have been deposited with ATCC, the deposit numbers and data being given on page 23 of the specification. As specifically stated there, the deposits have been made under the Budapest Treaty and samples of the deposited plasmid will become available to

public upon grant of a patent on this US Patent application. A copy of the deposit form is enclosed.

It is hereby stated, under the signature of the undersigned, that all restrictions to public access to the deposits will be removed upon the grant of a patent on this application and that non-viable deposits will be replaced. It is submitted that claim 7 is not open to rejection under 35 USC 112, first paragraph.

Claim 8 defines plasmid pQWE and pQWF and refers to Figures 19 and 20 for illustrations of these plasmids. These plasmids have not been deposited since the specification provides sufficient information to enable a person skilled in the art to produce such plasmids. In this regard, as seen in Figure 19, plasmid pQWE is derived from deposited plasmid pKS348. The steps required to assemble pQWE from pKS348 are shown diagrammatically in Figure 19. As is clear from the specification, pQWE differs from pKS348 solely in that pQWE contains a C-terminal fragment of the 200 kDa gene. As seen in Figure 19, this is achieved by cutting out a *Nde* I/*Nae* I fragment from pKS348, which is an approximately 5.8 kb fragment containing the T7 promoter, ampicillin antibiotic resistance marker and the 3' end of the 200 kDa gene (see description in Example 15). The other 480 bp fragment was restriction digested with *Nla* and *Pst* I and the resulting *Nde* I/*Nla* IV fragment ligated with the previously isolated 5.8 kDa *Nde* I/*Nae* I fragment to produce plasmid pQWE. It is clear, therefore, that the process steps are fully described whereby pQWE can be obtained from deposited pKS348. There is no necessity, therefore, for a deposit of plasmid pQWE.

With respect to pQWF, this plasmid is derived directly from both pKS348 and pQWE. In this regard, reference is made to Figure 20 and Example 15. In this case, an approximately 500 bp fragment from the *Eco*RI site in the 200 kDa gene in pKS348 was PCR amplified using oligos 6425.KS and 4272.KS, both of which are identified by nucleotide sequence in Table 10. The resulting approximately 500 bp PCR fragment was then restriction digested with *Nde* I and *Eco*RI.

Plasmid pQWE, prepared as described above and illustrated in Figure 19, was restriction digested with *Nde* I and *Eco*RI. The *Nde* I/*Eco*RI PCR fragment

then was ligated into the *Nde* I/*Eco*RI fragment from pQWE to produce pQEF. It is clear, therefore, that the process steps are described whereby pQWE can be obtained from deposited pKS348. There is no necessity, therefore, for a deposit of plasmid pQWF.

Having regard to thereto, it is submitted that claims 7 and 8 cannot be considered to lack enablement and hence the rejection thereof under 35 USC 112, first paragraph, should be withdrawn.

The Examiner rejected claims 1 to 6, 9 and 10 under 35 USC 102(e) as being anticipated by Sasaki et al. Having regard to the following discussion, it is submitted that the reference does not anticipate any of the applicants claims.

Applicants claim in claim 1 an isolated and purified nucleic acid molecule having a defined nucleotide sequence. The nucleotide sequence may be (a) a nucleotide sequence consisting of SEQ ID No: 5, 6, 8 or 10, (b) a nucleotide sequence encoding an about 200 kDa outer membrane protein of a strain of *Moraxella* and having derived amino acid sequence consisting of SEQ ID No: 7, 9 or 11, or (c) a nucleotide sequence encoding an about 200 kDa outer membrane protein of another strain of *Moraxella* (i.e. besides 4228, Q8 and LES-I shown in (a) and (b)) which is characterized by:

- a tract of consecutive G nucleotides which is 3 or a multiple thereof in length

- an ATG start codon about 80 to 90 bp upstream of the tract

- the tract being located between amino acids 25 and 35 encoded by the nucleotide sequence.

It is submitted that such sequences are not shown in Sasaki et al and hence claim 1 cannot be considered to be anticipated by this reference. The Examiner asserts in the Office Action that:

> "Sasaki et al disclose an isolated and purified nucleic acid molecule having a nucleotide sequence encoding an outer membrane protein of a strain of *M. catarrhalis*, which is characterized by a tract of consecutive 3 base-long G nucleotides and an ATG start codon as

> recited, and a plasmid or an expression vector for transforming a host comprising the nucleic acid molecule; the host cell is *E. coli* (see abstract; sequence listing; Examples 6, 10 and 11; and paragraph bridging columns 2 and 3)."

First of all, it must be observed that Sasaki et al gives only a nucleotide sequence for nucleic acid isolated from *M. catarrhalis* strain 4223 but identifies no open reading frame with respect to the disclosed sequence and no start codon. There is not described in Sasaki "an ATG start codon as recited" as asserted by the Examiner. It is not seen in what manner that Sasaki et al can be considered to possess "a tract of consecutive 3 base-long G nucleotides" as asserted by the Examiner and, in particular, the relationship defined in claim 1(c).

As set forth in the specification, the Sasaki et al sequence was derived from a nucleic acid molecule in a *M. catarrhalis* strain 4223 λ EMBL3 clone (see Examples 9 and 10 of Sasaki et al). The open reading frame is identified in WO 96/34960, of record therein, (see Figure 6 herein) and is reproduced as Figure 2 of this application. The open reading frame was predicted to start at a GTG codon. This molecule sequence has a tract of 10 nucleotides upstream from the GTG start site.

However, when sequence analysis was performed on 4223 genomic PCR-amplified subclones, as described herein, a longer open reading frame was found starting from an ATG start codon with a G-tract of 9 nucleotides downstream of the ATG start codon in the chromosomal gene. The nucleotide sequence and the derived amino acid sequence are shown in Figure 3 of this application. For the Examiner's convenience, we enclose a copy of Figures 6A and B of Sasaki et al which has the G tract and GTG start codon highlighted. The ATG sequence which became the start codon in the chromosomal gene also is marked thereon. It is again emphasized that Sasaki et al itself does not identify in Figure 6 an open reading frame or a start codon. To the extent there is a tract of G nucleotides, the number of G's in the tract is 10 in Sasaki et al. It would appear, therefore, that an addition G nucleotide had been inserted during cloning of the gene by Sasaki et al from the phage library. It is recognized that there is a tract of 3 G nucleotides at around na 650 but, again, no open reading frame is described. It will be noted that, in Figure 3

of this application, this codon is outside the parameters of location of the G tract recited in claim 1(c).

As applicant notes in the specification, analysis of the 5' end of the 200 kDa gene from 24 strains of *M. catarrhalis* suggests that the number of G nucleotides in the G tract acts as a regulator of expression. In this regard, reference is made to Table 5.

The nucleotide sequence from strain 4223 as set forth in Figure 3, (SEQ ID Nos. 5 and 6) is not disclosed in Sasaki et al and neither is the nucleotide sequence for strain Q8 (Figure 4; SEQ ID No: 8) and LES-I (Figure 5; SEQ ID No: 10) (Claim 1(a)). In addition, the derived amino acid sequences from these strains as set forth in Figures 3, 4 and 5 (SEQ ID Nos: 7, 9 and 11) are not disclosed in Sasaki et al (claim 1(b)). Furthermore, Sasaki et al does not disclose a nucleic acid molecule having the parameters of claim 1(c).

It is clear, therefore, that claim 1 is not anticipated by Sasaki et al nor claim 2, claim 5 when dependent on claim 1 or 2, claim 6, claim 9 and claim 10.

With respect to claim 4, this claim is clearly not anticipated by Sasaki et al. This claim defines an isolated and purified nucleic acid molecule which has SEQ ID No: 5. The only fragments of the full length described in Sasaki et al and the fragments contained in plasmid pKS5, pKS47 and pKS9 (see Figure 5), which are not the same as the claimed fragment.

Claim 3 defines an isolated and purified nucleic acid molecule having a nucleotide sequence which is (a) a nucleotide sequence having SEQ ID No: 12, which is a specific 5' truncation of the full length gene, (b) a nucleotide sequence encoding the derived amino acid and sequence consisting of SEQ ID No: 13, which is a specific N-terminal truncation of the full length protein, or (c) a corresponding nucleotide sequence encoding a 5'-truncation of a gene encoding an about 200 kDa outer membrane strain of another strain of *M. catarrhalis* i.e. other than 4223, Q8 and LES-I. The strain must be one which expresses the N-terminally truncated OMP from *E. coli*.

Since the Sasaki et al reference does not describe a nucleic acid as defined in claim 3(a) and (b), it follows that Sasaki et al does not anticipate claim 3(c). Claim 5 when dependent on claim 3 and claims 6, 9 and 10 similarly are not anticipated.

It is submitted, therefore, that claims 1 to 6, 9 and 10 are not anticipated by Sasaki et al and hence the rejection thereof under 35 USC 102(c) thereover, should be withdrawn.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

Michael I. Stewart
Reg. No. 24,973

Toronto, Ontario, Canada,
(416) 595-1155
FAX No. (416) 595-1163

Appl. No. 09/361,619

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 13 of page 9 has been amended as follows:

Figure 13 shows the SDS-PAGE analysis of the purification of the M56 r200 kDa protein according to the scheme of Figure 11. Lane 1, *E. coli* whole cells; Lane 2, soluble proteins after 50 mM Tris/NaCl, pH8, extraction; Lane 3, soluble proteins after Tris/Triton X-100®/EDTA extraction; Lane 4, soluble proteins after Tris/OG extraction; Lane 5, pellet after Tris/OG extraction; Lanes 6, 7, purified 200 kDa protein;

Paragraph beginning at line 4 of page 28 has been amended as follows:

The 5' region of the 200 kDa protein gene was amplified from strain 4223 chromosomal DNA. PCR reactions were performed using Taq Plus or Tsg Plus enzyme (Sangon Ltd., Scarborough, Ont., Canada) and a Perkin Elmer DNA Thermocycler (Perkin Elmer Cetus, Foster City, CA, USA). The lower PCR reaction mixture (50 μl) contained 5 μl of 10X buffer, 0.4 mM each of four deoxynucleotide triphosphates (Perkin Elmer, Foster City, CA, USA) and 1 to 2 μM each of two primers. The upper PCR reaction mixture (50 μM) contained 5 μl of 10X buffer, 0.5 to 1 μl of Taq Plus or Tsg Plus enzyme, and template DNA. The lower and upper mixtures were separated by a layer of AmpliWax PCR Gem50® (Perkin Elmer, Foster City, CA, USA) before heating cycles started. The thermocycling condition employed for the provision of PCR products in the construction of various plasmids are set forth in Table 11 below. The PCR products were purified using a QIAquick PCR purification kit (Qiagen Inc., Mississauga, Ont., Canada). The purified PCR products were sequenced on both strands directly and/or after cloning in appropriate vectors using an Applied Biosystem sequencer.

Paragraph beginning at line 28 of page 31 has been amended as follows:

The deletion of a short 5' region from the 4223 200 kDa protein gene is shown in Figure 10 and was performed using a similar approach as described in Example 4. An about 500 bp 5' region of the 200 kDa gene was PCR amplified from strain 4223 using primers 5471.KS and 4257.KS (Table 8) from chromosomal DNA. The 5' primer (designated 5471.KS) was based upon the region surrounding the previously identified GTG downstream start codon. In primer 5471.KS, the flanking regions around the GTG codon were incorporated and the GTG was mutated to ATG with further mutations used to introduce an *Nde*I site incorporating the new ATG. Using numbering from the full-length 200 kDa protein, the new start codon would be M56 replacing the previous V56 codon. The 3' primer (designated 4257.KS) was based upon the non-coding strand located about 500 bp downstream from the GTG codon in the 200 kDa protein gene. The PCR-product was digested with *Nde*I, purified using a QIAquick® PCR purification kit (Qiagen Inc., Mississauga, Ont.), and inserted into *Nde*I digested and dephosphorylated pKS122 to provide pKS348 (see Figure 7). Plasmid pKS348 was confirmed by restriction enzyme analyses and by sequencing of the PCR-amplified DNA piece and its joint regions. The nucleotide sequence (SEQ ID No: 12) and the deduced amino acid sequence (SEQ ID No: 13) for the 5'-truncation contained in pKS348 are shown in Figure 8. A similar N-terminal truncated 200 kDa gene from strain LES-1 was generated in the same manner and was designated pKS444.

Paragraph beginning at line 20 of page 33 has been amended as follows:

E. coli cell pellets were obtained from 500 ml culture prepared as described in Example 7, by centrifugation and were resuspended in 50 ml of 50 mM Tris-HC1, pH 8.0, containing 0.1 M NaCl, and disrupted by sonication. The sonicate was centrifuged at 20,000 xg for 30 min. and the resultant supernatant (sup1) was discarded. The pellet (ppt1) was extracted, in 50 ml of 50 mM Tris-HC1, pH 8.0 containing 0.5% Triton X-100® and 10 mM EDTA, then centrifuged at 20,000 xg for 30 min. and the supernatant (sup2) was discarded. The pellet (ppt2) was further extracted in 50 ml of 50 mM Tris-HC1, pH 8.0, containing 1% octylglucoside, then centrifuged at 20,000 xg for 30 min. and the supernatant (sup3) was discarded.

Paragraph beginning at line 34 of page 33 has been amended as follows:

The resultant pellet (ppt3) contained the inclusion bodies. The pellet was solubilized in 6 ml of 50 mM Tris-HC1, pH 8.0, containing 6 M guanidine and 5 mM DTT. Twelve ml of 50 mM Tris-HCl, pH 8.0 was added, the mixture centrifuged at 20,000 xg for 30 min, and the pellet (ppt4) discarded. The supernatant (sup4) was precipitated by adding polyethylene glycol (PEG) 4000 at a final concentration of 5% and incubated at 4°C for 30 min. The resultant pellet (ppt5) was removed by centrifugation at 20,000 xg for 30 min. The supernatant was then precipitated by $(NH_4)_2SO_4$ at 50% saturation at 4°C overnight. After the addition of $(NH_4)_2SO_4$, the solution underwent phase separation with protein going to the upper phase (as judged by the cloudiness of the layer). The upper phase was collected, then subjected to centrifugation at 20,000 xg for 30 min. The resultant pellet was collected and dissolved in 2 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine and 5 mM DTT. The clear solution was purified on a Superdex 200® gel filtration column equilibrated in 50 mM Tris-HC1, pH 8.0, containing 2 M guanidine HC1. The fractions were analysed by SDS-PAGE and those containing the purified r200 kDa were pooled. The pooled fraction was concentrated 5 to 10 fold using a centriprep 30 and then dialysed overnight at 4°C against PBS, and centrifuged at 20,000 xg for 30 min to clarify.

Paragraph beginning at line 20 of page 37 has been amended as follows:

Twenty-five µl of diluted pre-immune serum or test antiserum were added to the wells of a 96-well Nunclon® microtitre plate (Nunc, Roskilde, Denmark). Twenty-five µl of diluted bacterial cells were added to each of the wells. A guinea pig complement (BioWhittaker, Walkerville, MD) was diluted 1:10 in VBS, and 25 µl portions were added to each well. The plates were incubated for 60 min, gently shaking at 70 rpm on a rotary platform. Fifty µl of each reaction mixture were plated onto Mueller Hinton agar plates (Becton-Dickinson, Cockeysville, MD). The plates were incubated at 37°C for 24 hours, and then left at room temperature for a further 24 hours. The number of colonies per plate was counted, and average values of colonies per plate were estimated from duplicate pairs.